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Role of Platelets and Fibrin in the Healing Sequence

An In Vivo Study of Angiogenesis and Collagen Synthesis

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The signals that initiate repair are poorly characterized. These studies investigate the capacity of platelets and fibrin to initiate angiogenesis, fibroplasia, collagen synthesis and monocyte migration in the rabbit cornea assay. Methods: Autologous platelets and platelet-free fibrin were isolated from rabbit blood. Released and control platelet preparations and autologous and commercial fibrin were implanted in rabbit corneas. Results: Thrombin-released platelets produced angiogenesis and opacification. Histology showed fibroplasia, corneal thickening, and neovascularization. Collagen synthesis was elevated to twice control levels in thrombin-activitated platelet preparations. Various control platelet preparations produced no angiogenesis, no opacification, and no histologic change. All fibrin injections elicited a cellular exudate from the limbal vessels, followed by angiogenesis and corneal opacification. Histology showed a mononuclear infiltrate with neovascularization and fibroplasia. Control injections of rabbit skin collagen and fibroblasts produced no response.

ANGIOGENESIS (the proliferation and directed growth of capillary endothelium), fibroplasia, and collagen synthesis are integral components of the host's response to wounding. Normal repair follows an orderly sequence of cellular events which results in new connective tissue formation. Resting fibroblasts at the wound edge are stimulated to divide, migrate, and produce collagen. Capillary endothelial migration and division from venules and capillaries at the wound edge construct new capillary loops that provide nutrients for the increased cellular activity. Fibroplasia and collagen synthesis begin within 24 hours of wounding, followed in 48–72 hours by endothelial migration and mitosis.[1]

The signals that initiate this healing response are largely uncharacterized. It is reasonable to suspect that the products of coagulation could play a role in the cellular response to wounding, since activiation of platelets and the clotting cascade are among the first reactions to injury.

Platelets activated by thrombin release a mitogen for fibroblasts and smooth muscle cells and stimulate increased collagen synthesis by smooth muscle cells *in vitro*. This mitogen (platelet-derived growth factor, or PDGF) has been isolated and partially characterized.

Fibrin, the end product of the coagulation cascade, and fibrin degradation products are chemotactic for leukocytes *in vitro*. Neutrophils are the predominant inflammatory cell in the traumatic central dead space of the wound from the first to the third day following injury. However, macrophages gradually replace the neutrophils three to four days after wounding and remain in the central dead space of the wound until repair is complete. Macrophages produce growth factors that stimulate neovascularization, fibroblast proliferation, and migration.

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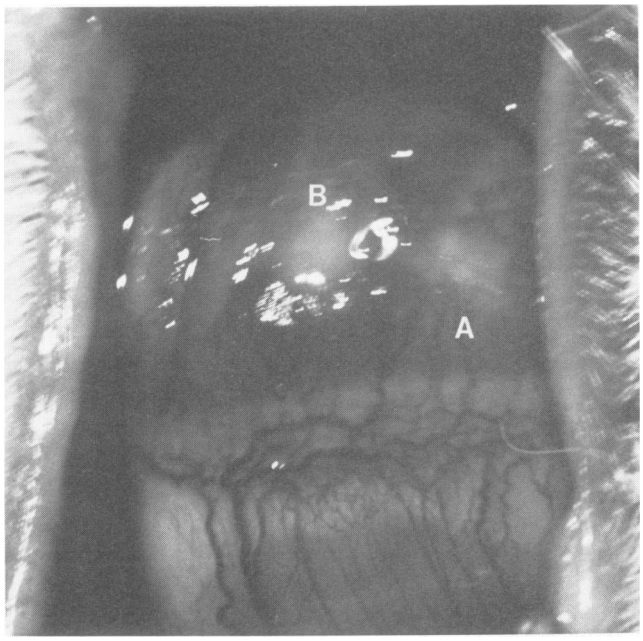

FIG. 1. Rabbit cornea implanted with 1×10^9 thrombin-activated platelets. A 4+ angiogenic response to the implanted platelets is marked A. Corneal opacification over the injected platelets is marked B.

This study utilizes the rabbit cornea assay to determine (1) whether the products of activated platelets produce neovascularization and collagen synthesis in an *in vivo* assay system, and (2) the role of fibrin in eliciting macrophage migration and subsequent angiogenesis *in vivo*.

Materials and Methods

Platelet-Derived Angiogenesis

To determine whether autologous platelets could produce angiogenesis *in vivo*, the rabbit cornea assay was utilized in female New Zealand White rabbits. Autologous rabbit platelets were isolated from 10–35 ml of blood obtained by cardiac puncture. The blood was collected in siliconized tubes containing acid-citrate dextrose (0.15 M citrate, 2% glucose, pH 4.2) and was centrifuged at 900 rpm for 20 minutes. The platelet-rich plasma was removed and centrifuged at 2500 rpm for 20 minutes and resuspended in HEPES buffer solution (0.2 M HEPES, 0.03 M glucose, 0.004 M KCl, 0.14 M NaCl, 0.35% bovine serum albumin pH 6.5) to the desired concentration.

Four different platelet preparations were assayed for their angiogenic capacity: (1) nonactivated platelets (8×10^7) were suspended in 0.05 ml HEPES buffer solution (pH 7.2) and injected into nine corneas; (2) thrombin (1 U/ml)-activated platelets (8×10^7) were injected into 21 corneas; (3) thrombin-activated, washed platelets (8×10^7) were injected into four corneas; (4) thrombin-activated, washed, thrombin-re-enriched platelets (8×10^7) were injected into four corneas.

A dose-response curve for thrombin-activated platelets was constructed by injecting eight corneas with 1×10^7 platelets, eight corneas with 1×10^8 platelets, and five corneas with 1×10^9 platelets.

The corneal injections were carried out in rabbits anesthetized with IV pentobarbital. The corneas were anesthetized with 1% ophthalmological xylocaine. The platelets were suspended in 0.05 ml of buffer solution and injected into the corneas with a 30-gauge hypodermic needle and a 1 ml syringe. The material was injected approximately 3 mm from the limbus of the cornea. The corneas were inspected every other day for the onset and intensity of the neovascular response and the amount of corneal opacification. Representative corneas were examined histologically and photographed. Neovascularization was graded from 0 to +4. No growth of new vessels into the cornea was graded 0; growth of vessels 1 mm into the corneal stroma in a localized arc just adjacent to the implant was graded +1; 2–3 mm ingrowth of vessels in a localized arc turned toward the implant was graded +2; 2–3 mm ingrowth into the cornea with a widening arc of vessels from the limbus was +3; and vessel growth greater than 3 mm into the cornea associated with a wide arc of neovascularization toward the implant was graded +4.

Platelet-Derived Collagen Synthesis

To determine whether autologous, thrombin-activated platelets stimulate collagen synthesis in the rabbit cornea, platelets were obtained as described above and activated with thrombin (1 U/ml). Six corneas were injected with 1×10^8 platelets. Eight days after injection, the corneas were inspected for the degree of opacification and angiogenic response. The cornea at the injection site was removed from each eye using a corneal punch (6 mm diameter). Control corneas were injected with 0.05 ml buffer solution, and the area of injection was removed in a similar manner.

The corneas were then incubated for 13 hr at 37 C in 3 ml Krebs' Ringer's (phosphate-free) + 20 mM glucose + 20 mM HEPES buffer (pH 7.6) + 0.05 mg/ml ampicillin + 1 uCi C^{14}-proline. The reaction was terminated by freezing. After thawing, the sample was washed in buffer without proline, hydrolyzed in 6 N HCl, and hydroxyproline was isolated and counted in a scintillation counter. Results are expressed as net counts per minute (cpm)/sample.[2,3] Statistical analysis was performed using unpaired Student's t-test.

The Role of Fibrin in Repair

Two different methods of producing fibrin were used to measure the *in vivo* response to fibrin implantation

in the rabbit cornea. Homologous rabbit fibrin clot was produced by collecting 35 ml of rabbit blood by cardiac puncture. The blood was collected in acid-citrate-dextrose solution and centrifuged at 900 rpm for 20 minutes. The platelet-rich plasma was then removed and centrifuged at 2000 rpm for 20 minutes. The platelet-poor plasma was removed and centrifuged at 20,000 rpm for 20 minutes. The resulting platelet-free plasma was recalcified to 17–21 mM with 1 M $CaCl_2$ and heated to 56 C for 30 minutes to ensure complete polymerization. The clot was removed by centrifugation, washed five times with phosphate-buffered saline (PBS) and lyophilized.

Fibrin was also produced by adding 1 U thrombin to purified bovine fibrinogen (5 mg) in 5 ml PBS. The solution was heated to 56 C for 30 minutes and the resulting fibrin clot removed by centrifugation. The fibrin was washed five times with PBS and lyophilized.

Both fibrin preparations were implanted into corneal pockets for assay. The dome of the corneas was incised with a knife and a corneal spatula was used to fashion a pocket in the lamellae of the cornea. The pocket ended 1–2 mm from the limbus. Ten corneas were implanted with approximately 0.5 mg homologous fibrin. Ten corneas were also implanted with approximately 0.5 mg commercial bovine fibrin. The eyes were inspected every other day, and the resulting response from the rabbit limbus was monitored. Representative corneas were examined histologically. Four corneas were implanted with 0.5 mg purified rabbit skin collagen and four with bovine albumin as controls.

TABLE 1. *Thrombin-Activated Platelets*

Number of Corneas Implanted	Number of Platelets Injected	Angiogenesis (0–4+)	Corneal Opacification
5	1×10^9	4+	3–4 mm
8	1×10^8	2+–3+	1–2 mm
21	8×10^7	2+–3+	1–2 mm
8	1×10^7	1+–2+	0–1 mm
	Unactivated Platelets		
9	8×10^7	0	0
	Thrombin-activated, Washed Platelets		
4	8×10^7	0	0
	Thrombin-activated, Washed, Thrombin Added		
4	8×10^7	0	0

Results

Platelet-Derived Angiogenesis

Autologous thrombin-activated platelets produced neovascularization characterized by ingrowth of capil-

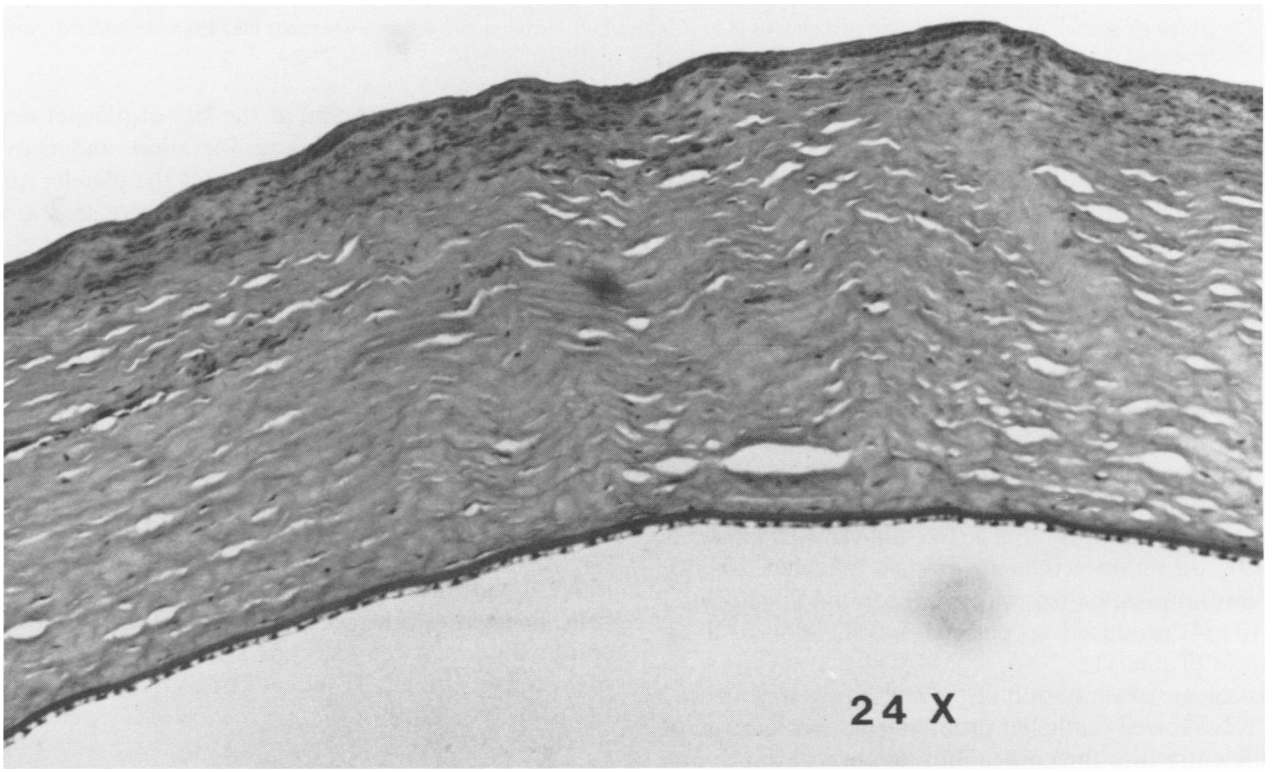

FIG. 2. Histologic section of a contral platelet injection (8×10^7 nonactivated platelets). Note the normal corneal architecture with no corneal thickening or neovascularization.

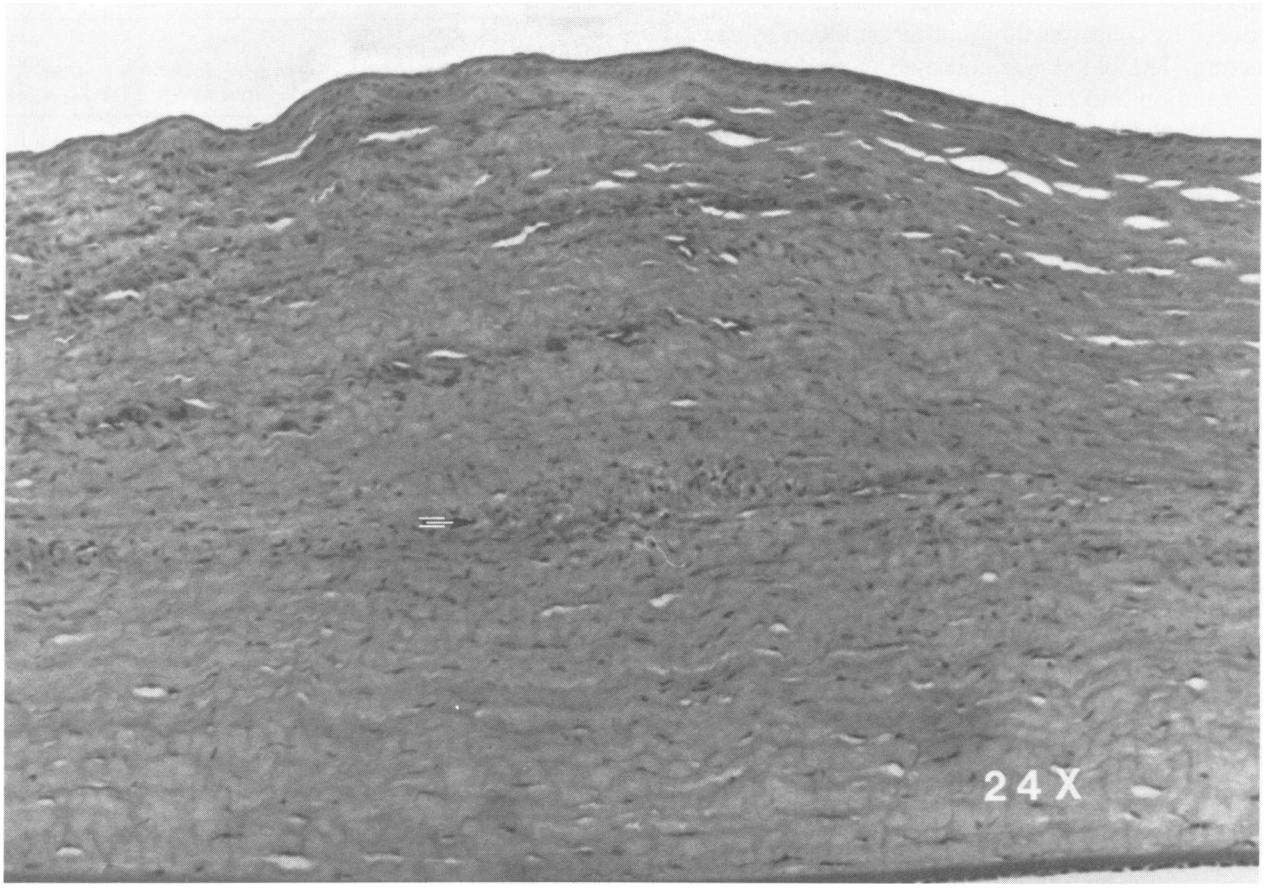

FIG. 3. Histologic section of a thrombin-activated platelet (8×10^7) injection. Arrow shows the area where the platelets were injected. Note the thickening of the cornea to almost twice the normal diameter.

lary sprouts into the cornea toward the injection site in 48–96 hours after implantation. Thrombin-activated platelets also produced corneal opacification 48–72 hours after injection. The corneal responses were dose related; 1×10^7 thrombin-activated platelets produced less than 1 mm corneal opacification and 1+–2+ angiogenesis, 8×10^7 platelets produced 1–2 mm corneal opacification and a 2+–3+ angiogenic response (19+/21), 1×10^8 platelets produced 2–3 mm corneal opacification and a 2+–3+ neovascular response (8+/8), and 1×10^9 thrombin-activated platelets produced 3–4 mm corneal opacification and 4+ angiogenic response (5+/5) (Fig. 1).

Control injections of 8×10^7 unactivated platelets (1+/9), thrombin-activated, washed platelets (0+/4), and thrombin-activated, washed, thrombin-added platelets (0+/4) produced no corneal opacification or angiogenesis (Table 1).

Histologic examination of the corneas in unactivated controls showed epithelial proliferation over the site of needle entry into the cornea, but no corneal thickening or neovascularization was present (Fig. 2). Corneas injected with thrombin-activated platelets showed epithelial proliferation over the site of needle entry into the cornea, corneal thickening at the site of platelet deposition with active fibroblast proliferation, and neovascularization from the limbus towards the platelet injection site. Very few inflammatory cells were seen at the site of platelet injection (Fig. 3).

Platelet-Derived Collagen Synthesis

All corneas injected with 5×10^8 thrombin-activated platelets (+6/6) produced neovascularization and corneal opacification. Control corneas injected with buffer solution produced no neovascularization or opacification. Collagen synthesis measured using the Uitto method ranged from 555–901 cpm, with a mean of 714 cpm ± 137 standard deviation of the mean, in corneas injected with thrombin-activated platelets. Control corneas, injected with buffer alone had a range of 416–464 cpm, with a mean of 431 cpm ± 23 standard deviation of the mean. Unpaired Student's t-test analysis produced $t = 5.74$, $p < 0.001$.

The Role of Fibrin in Repair

Corneas implanted with fibrin, regardless of whether the fibrin was autologous or commercially produced,

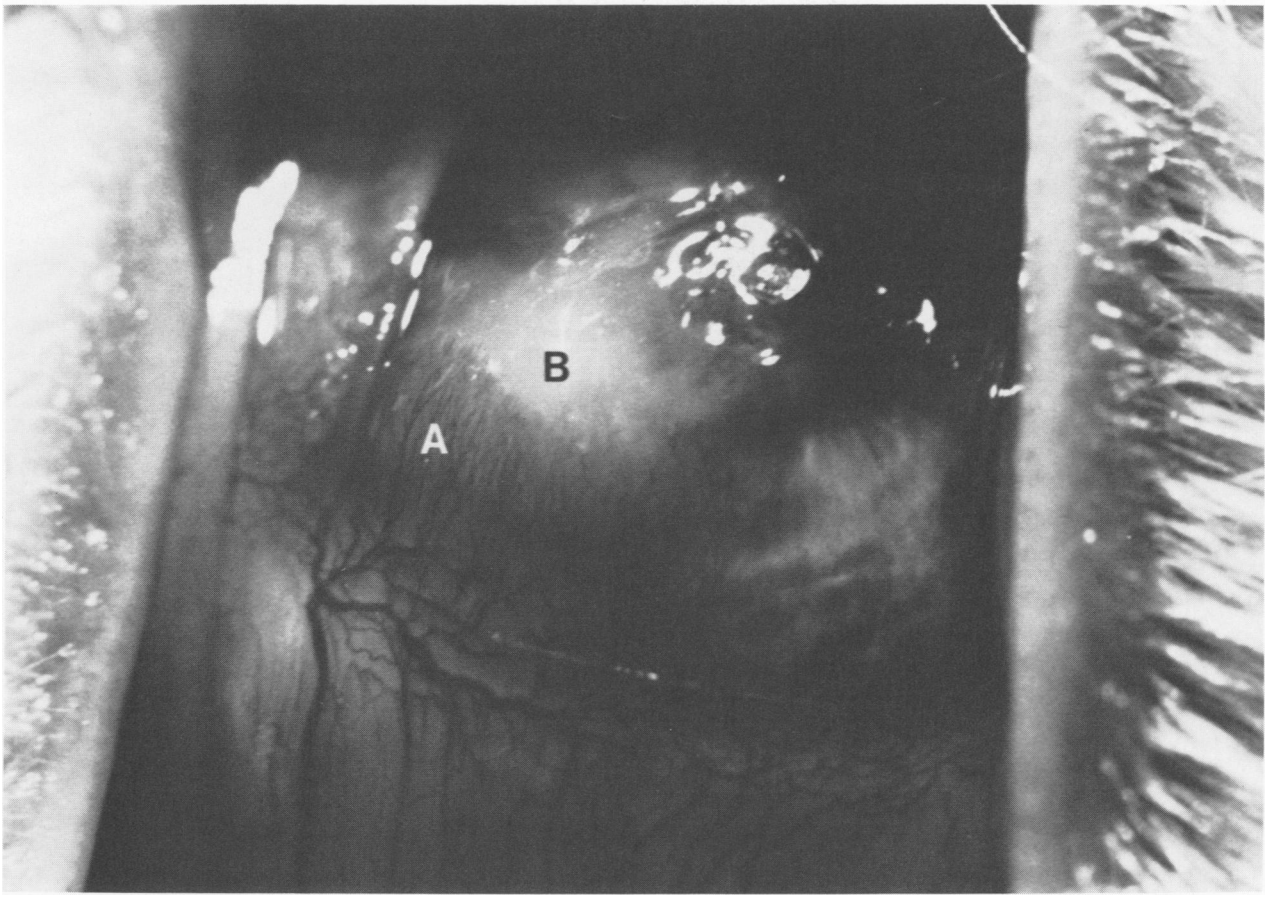

FIG. 4. Rabbit cornea implanted with commercially purified bovine thrombin. A 4+ angiogenic response from the limbus is marked A. B marks the site of fibrin implantation with resulting corneal opacification.

showed similar responses. Two days after implantation there was a cellular response from the limbus, which progressed over the next two days toward the implanted fibrin. The cells leaving the limbal vessels produced corneal opacification as they proceeded in a front toward the pocket. This influx of cells was followed by intense corneal neovascularization and opacification (Fig. 4.

Histologically, the cellular response from the limbus consisted of neutrophils, macrophages, and plasma cells. There was intense neovascularization and fibroblast proliferation with corneal thickening (Fig. 5).

Control implantations of rabbit skin collagen or bovine albumin produced no corneal opacification or angiogenesis, and appeared histologically as normal corneas without thickening or neovascularization (Fig. 6).

Discussion

These experiments demonstrate that thrombin-activated platelets have the capacity to stimulate angiogenesis and increased collagen synthesis, as measured in the *in vivo* rabbit corneal assay, and that fibrin, fibrinopeptides and/or fibrin degredation products produce leukocyte migration into the cornea with subsequent neovascularization and corneal opacification. Appropriate platelet controls show that the active factor is released by the thrombin-activated platelet and not caused by the exposure of the unactivated platelet to the corneal stromal collagen, by the presence of platelet ghosts in the cornea, or by the action of thrombin alone. Implantation of collagen and albumin into the cornea demonstrates that native proteins other than fibrin produce no corneal reaction.

Previously, the study of platelet-derived growth factors in disease and repair has focused on their effects on smooth muscle cells, fibroblasts, and umbilical and aortic endothelial cells in culture. Ross and coworkers first focused attention on the platelet as a source of growth factor by showing that the growth stimulating activity in serum for smooth muscle cells and fibroblasts was abolished by carefully removing the platelets and was present when the supernatant from thrombin-activated platelets was added to the platelet-poor plasma.[4,5] Since that time platelet derived growth factor (PDGF) has been isolated and characterized as two glycoproteins with molecular weights of 31,000 and 28,000 daltons.[6-8] Some controversy remains with regard to the size and active fraction of the isolates.

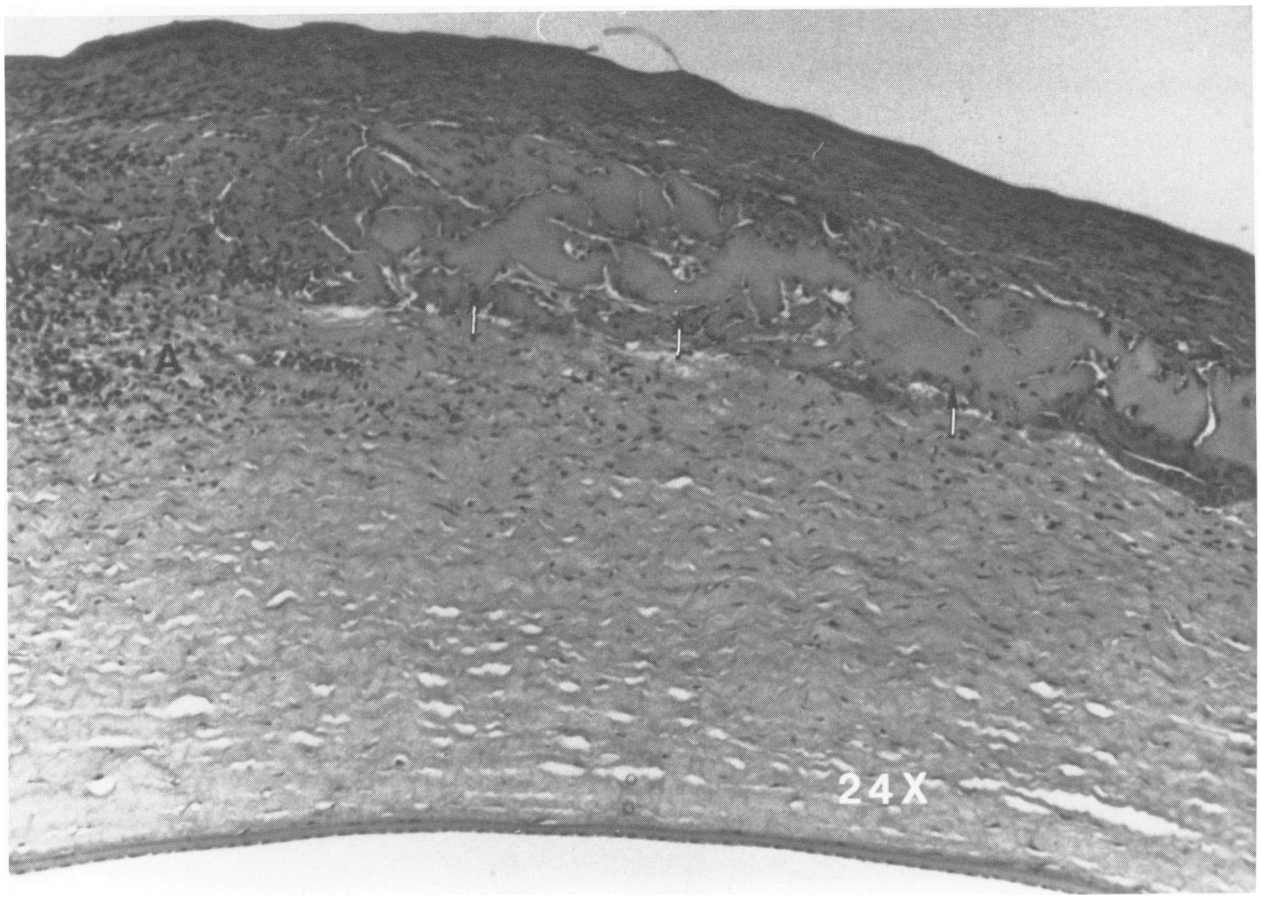

FIG. 5a. Histologic section of a corneal implanted with bovine fibrin. Arrows indicate implanted fibrin. Cellular response to the fibrin is marked with the letter A.

The action of PDGF *in vitro* has been studied extensively. In addition to its mitogenic properties, PDGF is chemotactic for cultured fibroblasts,[9] and produces increased collagen synthesis from cultured, growth-arrested myocytes and fibroblasts.[10,11] Receptors for PDGF have been demonstrated on skin fibroblasts, normal and malignant glial cells, smooth muscle cells, and 3T3 cells. Receptors were not found on epithelial-derived cells, neuroblastoma cells or human umbilical vein endothelial cells.[12]

The effect of PDGF on endothelial cell mitosis and migration has been studied using umbilical vein and aortic arch cells. Wall et al. demonstrated that umbilical vein endothelial cells migrate in response to PDGF but would not respond by an increase in cellular division.[13,14] Zetter, on the other hand, demonstrated that umbilical cells responded to partially purified PDGF with a modest increase in cell number when cultured in platelet-poor plasma, while the combination of PDGF and thrombin produced a log increase in cell number after eight days.[15] Robertson was unable to show either an increase in cell number or migration in response to PDGF.[16] D'Amore showed that preconfluent aortic arch endothelial cells respond to PDGF with increased rates of cell division.[17]

The authors' experiments show that factors released from thrombin-activated platelets produced angiogenesis *in vivo*. Ausprunk, in an electron microscopic study of capillary proliferation in the cornea, showed that angiogenesis is a combination of capillary endothelial cell migration with subsequent mitosis.[18] The results, therefore, predict that factor(s) from thrombin-released platelets should produce capillary endothelial cell migration and mitosis in culture. Preliminary results from the authors' laboratory using cultured rabbit brain capillary endothelial cells and rabbit platelet release factor supports the *in vivo* observation.

The effect of PDGF on protein synthesis has been investigated using smooth muscle cells, fibroblasts and synovial cells. Castor et al. showed that factors from human platelets produced a seven-fold increase in glycosaminoglycans synthesis.[19] Burke et al. demonstrated an increase in collagen synthesis from cultured monkey smooth muscle cells,[10] and Malmquist et al. showed a

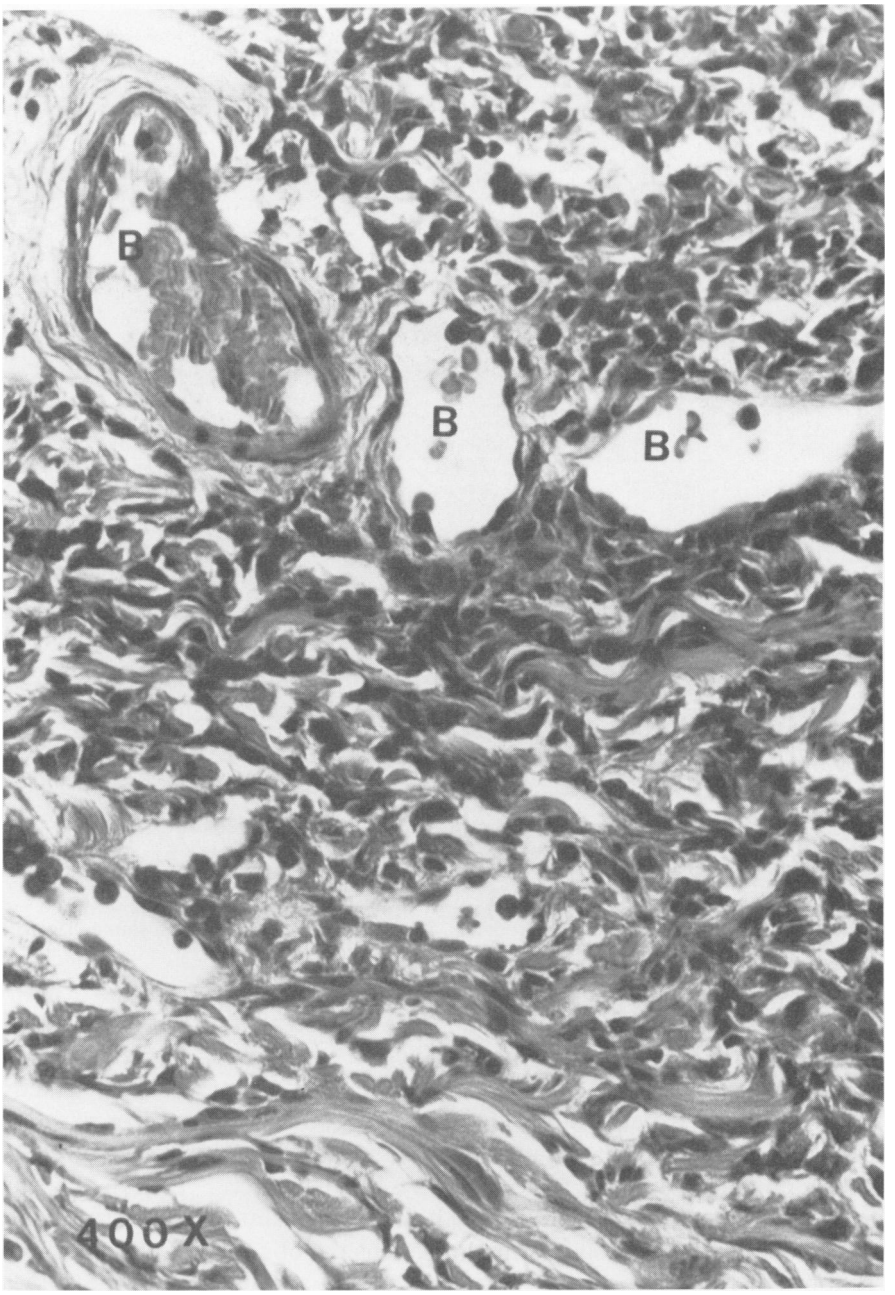

FIG. 5b. Higher power view of the cellular and angiogenic response to the implanted fibrin. Capillaries are marked with the letter B. Note the presence of inflammatory cells in the capillaries and also in the corneal stroma. Active fibroblast proliferation and collagen synthesis are also present.

two- to three-fold increase in collagen synthesis in cultured human and rabbit fibroblasts and aortic arch smooth muscle cells.[11] The authors' experiments extend these observations by showing that thrombin-activated platelets produce a statistically significant doubling of collagen synthesis *in vivo*. The actual rate of collagen synthesis in areas immediately adjacent to the injected platelets is probably even higher than that measured in the 6 mm corneal button, which included areas of relatively normal cornea around the injection site.

The classical view of fibrin in repair is that of an inert matrix that supports leukocytes and platelets and provides a substance on which wound cells can move or rest. A revision of this concept is forced by the authors' and other studies.

The role of fibrin in inflammation and repair has been studied previously. McKay reports that acute inflammation produced by bacterial endotoxin results in both intravascular and extravascular fibrin deposition, followed by leukostasis and migration of circulating leukocytes.[20] Colvin et al. also demonstrated intravascular and extravascular deposition of fibrin in delayed hypersensitivity reactions.[21] Kay et al. showed that the action of thrombin on fibrinogen produced a chemoattractant

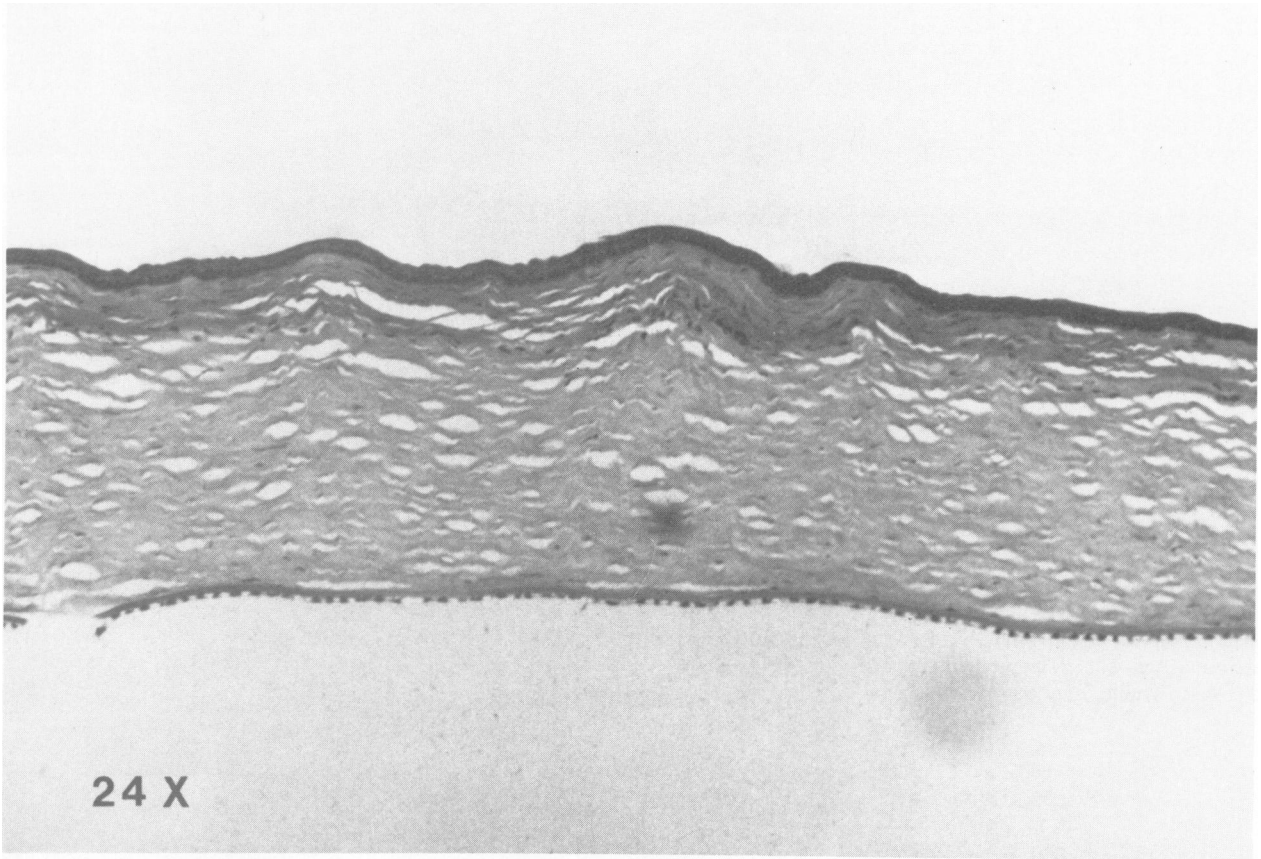

FIG. 6. Histologic section of a rabbit cornea implanted with purified rabbit skin collagen. There is no corneal thickening, angiogenesis, or cellular infiltrate.

for peripheral blood leukocytes and also showed that fibrinopeptide B (but not A) was an active chemoattractant.[22]

The authors' experiments show that fibrin, fibrinopeptides and/or fibrin degradation products produce leukocyte chemotaxis in an *in vivo* assay system. The fact that purified bovine fibrin produces a host response similar to that of homologous fibrin strengthens the observation that it is a product of fibrin production and/or degradation which is the active chemoattractant, and not fibronectin, platelets, or complement which could theoretically be implanted along with the fibrin prepared from blood.

The fact that an influx of inflammatory cells into the cornea is followed by intense neovascularization and fibroblast proliferation suggests that fibrin also acts as an activator of leukocyte production of growth and angiogenic factors. Previous experiments from this and other laboratories have shown that wound neutrophils do not produce neovascularization when injected into the rabbit cornea.[23] Wound macrophages, on the other hand, do produce angiogenesis and corneal opacification, enhance the rate of vessel formation in the rabbit ear chamber assay, and enhance smooth muscle cell and aortic arch endothelial cell growth in sparse cultures.[24–27] Activated peritoneal macrophages produce and/or release factors that result in angiogenesis, fibroblast proliferation, and migration, and enhance proliferation of aortic arch endothelial cells and smooth muscle cells.[28–33]

When examined in relation to the wound healing sequence, these results, along with other data, point to platelet release and production of fibrin as initiators of the cellular response to wounding. Platelet release could be responsible for the early burst of fibroplasia, collagen synthesis, and neovascularization observed at the wound edge. Fibrin and its degradation products, possibly along with complement chemoattractants, recruit tissue macrophages, and circulating monocytes to the wound site, and these cells, when stimulated, continue producing the necessary growth factors and chemoattractants until the repair is complete.

These findings predict that nonhealing wounds in patients with thrombocytopenia, leukopenia, and/or clotting disorders might be due to the absence of chemoattractant and mitogenic factors necessary for nor-

mal repair. Administration of systemic platelet transfusions, leukocyte transfusions, or frozen plasma should correct the deficit and initiate repair.

One patient, with thrombocytopenia and leukopenia secondary to myelofibrosis, developed an infected nonhealing pretibial wound after a biopsy. His steroids were decreased; he was placed on supplemental vitamin A and zinc; he was placed on supplemental oxygen, and given appropriate antibiotics and local wound care with Debrisan®. Regardless of these supportive therapies, no new granulation tissue formed in three weeks. Because of the authors' results in these experiments, he was then given a single, ten-unit, platelet transfusion. Three days later granulation tissue was observed at the base of the wound for the first time. The wound then proceeded to heal in three weeks by granulation, contraction, and epithelization.

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References

1. Hunt TK. Fundamentals of Wound Management in Surgery. Wound Healing: Disorders of Repair. South Plainfield, NJ: Chirugicom, 1976.
2. Peterkosky B, Prockop DJ. A method for the stimultaneous measurement of the radioactivity of proline-C^{14} and hydroxyproline-C^{14} in biological materials. Anal Biochem 1962; 4:400–406.
3. Uitto J. A method for studying collagen biosynthesis in human skin biopsy in vitro. Biochem Biophys Acta 1970; 201:438–445.
4. Rutherford RB, Ross R. Platelet factors stimulate fibroblasts and smooth muscle cells quiescent in plasma serum to proliferate. J Cell Biol 1976; 69:196–203.
5. Ross R, Glomset J, Kariya B, Harker L. A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc Natl Acad Sci USA 1974; 71:1207–1210.
6. Antoniodes HN, Scher CD, Stiles CD.Purification of human platelet-derived growth factor. Proc Natl Acad Sci USA 1979; 76:1809–1813.
7. Antoniodes HN. Human platelet-derived growth factor (PDGF): purification of PDGF-I and PDGF-II and separation of their reduced subunits. Proc Natl Acad Sci USA 1981; 78:7314–17.
8. Deuel TF, Huang JS, Proffitt RT, et al. Human platelet derived growth factor. Purification and resolution into two active protein fractions. J Biol Chem 1981; 256:8896–99.
9. Seppa H, Grotendorst G, Seppa S, et al. Platelet-derived growth factor is chemotactic for fibroblasts. J Cell Biol 1982; 921:584–88.
10. Stavenow L, Kellstrom T, Malmquist J. Stimulation of collagen production in growth-arrested myocytes and fibroblasts in culture by growth factor(s) from platelets. Exp Cell Res 1981; 136:321–25.
11. Burke JM, Ross R. Collagen synthesis by monkey arterial smooth muscle during proliferation and quiescence in culture. Exp Cell Res 1977; 107:387–95.
12. Helpin CH, Westermark B, Wasterson A. Specific receptors for platelet-derived growth factor on cells derived from connective tissue and glia. Proc Natl Acad Sci USA 1981; 78:3664–68.
13. Wall RT, Harker LA, Quadracci LJ, Striker GE. Factors influencing endothelial cell proliferation in vitro. J Cell Physiol 1978; 96:203–216.
14. Wall RT, Harker LA, Striker GE. Human endothelial cell migration—stimulation by a released platelet factor. Lab Invest 1975; 39:523–34.
15. Zetter BR, Antoniades HN. Stimulation of human vascular endothelial cell growth by a platelet derived growth factor and thrombin. J Supramol Structure 1979; 11:361–70.
16. Thorgeirsson G, Robertson AL. Platelet factors and the human vascular wall. Part 2. Such factors are not required for endothelial proliferation and migration. Antheroscerosis 1978; 31:231–38.
17. D'Amore P. Platelet–endothelial interaction and the maintenance of the microvasculature. Microvascular Res 1978; 15:137–145.
18. Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvascular Res 1977; 14:53–65.
19. Castor CW, Bignau MC, Hussler PA, Roberts DJ. Connective tissue activation XXI, regulation of glycosaminoglycon metabolism by lymphocyte (CTAP-I) and platelet (CTAP-III) growth factors. In Vitro 1981; 17:777–85.
20. McKay DG. Participation of components of the blood coagulation system in the inflammatory response. Am J Pathol 1972; 67:181–203.
21. Colvin RB, Johnson RA, Mihm Jr MC, Dvorak HF. Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. J Exp Med 1973; 138:686–698.
22. Kay AB, Pepper DS, Ewart MR. Generation of chemotactic activity for leukocytes by the action of thrombin on human fibrogen. Nature 1973; 243:56–57.
23. Simpson DM, Ross R. The neutrophil leukocyte in wound repair; a study with antineutrophil serum. J Clin Invest 1972; 51:2009–20.
24. Hunt TK, Andrews WS, Halliday B, et al. Coagulation and macrophage stimulation of angiogenesis and wound healing. Deneen P, ed. The Surgical Wound. Philadelphia: Lea & Febinger, 1982; 1–18.
25. Clark RA, Hunt TK. Role of macrophages in wound healing. Surg Forum 1976; 27:16–18.
26. Thakral KK, Goodson III WH, Hunt TK. Stimulation of wound blood vessel growth by wound macrophages. J Surg Res 1979; 30:430–436.
27. Greenburg GB, Hunt TK. The proliferative response in vitro of vascular endothelial and smooth muscle cells exposed to wound fluids and macrophages. J Cell Physiol 1978; 97:353–60.
28. Nathan CF, Murray HW, Cohn ZA. The macrophage as effector cell. N Engl J Med 1980; 303:622–26.
29. Unanue ER. Secretory function of mononuclear phagocytes. Am J Pathol 1976; 83:396–417.
30. Polverini PD, Cotran RS, Gimbrone MA, Unanue ER. Activated macrophages induce vascular proliferation. Nature 1977; 269:804–5.
31. Leibovich SJ. Production of macrophage-dependent, fibroblast-stimulating activity (M-FSA) by murine macrophages. Exp Cell Res 1978; 113:47–56.
32. Martin BM, Gimbrone MA, Unanue ER, Cotran RS. Stimulation of nonlymphoid mesenchymal cell proliferation by a macrophage-derived growth factor. J Immunol 1981; 126:1510–15.
33. Tsukamato Y, Helsel W, Wahl S. Macrophage production of fibronection, a chemoattractant for fibroblasts. J Immunol 1981; 127:673–678.